FUNDAMENTAL AND APPLIED TOXICOLOGY 36, 177–187 (1997)
ARTICLE NO. FA972296

Castel, SW et al. (1997)

Bioavailability of Lead to Juvenile Swine Dosed with Soil from the Smuggler Mountain NPL Site of Aspen, Colorado[1]

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Received October 30, 1996; accepted January 16, 1997

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Bioavailability of lead (Pb) has become an issue in quantifying exposure of sensitive populations and, where necessary, establishing cleanup levels for contaminated soil. Immature swine were used as a model for young children to estimate the degree to which Pb from two fully characterized composite samples from the Smuggler Mountain Superfund Site in Aspen, Colorado may be bioavailable to resident children. The composite soils contained 14,200 and 3870 μg Pb/g of soil. Relative and absolute enteric bioavailabilities of Pb in soil (oral dose groups of 75, 225, and 675 μg Pb/kg body wt/day) were estimated by comparison with an orally administered soluble Pb salt (lead acetate = $PbAc_2 \cdot 3H_2O$) (dose groups of 0, 75, and 225 μg Pb/kg body wt/day) and an intravenously administered aqueous solution of Pb (100 μg Pb/kg/day) from the same trihydrate salt administered daily for 15 days to 50 juvenile swine. The biological responses (area under the blood Pb concentration–time curve, and the terminal liver–, kidney–, and bone–lead concentrations) produced by Pb from $PbAc_2 \cdot 3H_2O$ and lead-contaminated soils were determined. This study revealed Pb from soil containing 14,200 μg Pb/g of soil had a bioavailability relative to Pb from PbAc (RBA), ranging from 56% based on the area under the blood lead concentration–time curve (AUC) versus dose, to 86% based on calculations from liver–Pb loading versus dose. Similarly, Pb from soil containing 3870 μg Pb/g of soil had an RBA ranging from 58% based on the AUC versus dose, to 74% based on calculations from liver– and kidney–Pb loading versus dose. Bioavailability of Pb in soils may be more or less than EPA's default RBA of 60%, therefore, measuring site-specific RBAs provides a basis for improved exposure and risk assessment.

Lead (Pb) poisoning in children is a serious, but preventable, environmental threat (USEPA, 1993). Continued concern with excessive exposure of young children and pregnant women to Pb results from epidemiologic studies indicating that low levels of Pb exposure can affect fetal and childhood development. Davis and Svendsgaard (1987) concluded that exposure to Pb levels sufficient to produce blood Pb (PbB) concentrations of 10 to 15 μg/dl and possibly lower were linked to undesirable developmental outcomes in human fetuses and children. A 1988 report to Congress stated that 3 million children in the United States have blood Pb concentrations above 15 μg/dl and that 4 million fetuses are estimated to be at risk for Pb toxicity during the next 10 years (USDHHS, 1988).

Centuries of mining, processing, and use have resulted in the redistribution of Pb in the environment, making it a ubiquitous multimedia contaminant. Lead-contaminated soil in and around urban areas remains a persistent problem (Rabinowitz and Bellinger, 1988; Aschengrau *et al.*, 1994). Of particular concern is the contamination of residential soils associated with past and present mining activities (LaVelle *et al.*, 1991; Cook *et al.*, 1993; Gulson *et al.*, 1994).

In general, the systemic availability of Pb is matrix and chemical species dependent (LaVelle *et al.*, 1991; Davis *et al.*, 1992; Gulson *et al.*, 1994; Mushak, 1991). Specifically, the gastrointestinal absorption of multimedia sources of Pb is influenced not only by physiological factors within the digestive tract (e.g., age, nutrition, and disease), but also by chemical and physical variables such as chemical species, particle size, and matrix association.

Incidental soil ingestion by young children is an important issue in assessing public health risks associated with exposure to lead-contaminated soils. Several studies have addressed this concern by providing estimates of the amount of soil ingested by children (Binder *et al.*, 1986; Clausing *et al.*, 1987; Calabrese *et al.*, 1989; Davis *et al.*, 1990; van Wijnen *et al.*, 1990).

Use of an appropriate animal model for investigating the

[1] Project funded by the United States Environmental Protection Agency Region VIII Office, Denver, Colorado.

0272-0590/97 $25.00

TABLE 1
Experimental Design for the Smuggler Mountain Superfund Site

Group	N	Treatment[a]	Acetate/soil lead[b]	Lead dose (μg Pb/kg-day) Target	Actual[c]
1	2	Vehicle control	Vehicle only	0	0
2	5	$Pb(Ac)_2 \cdot 3H_2O$	Weight adjusted	75	77
3	5	$Pb(Ac)_2 \cdot 3H_2O$	Weight adjusted	225	224
4	5	Berm soil	Mass and weight adjusted	75	76
5	5	Berm soil	Mass and weight adjusted	225	229
6	5	Berm soil	Mass and weight adjusted	675	732
7	5	Residential soil	Mass and weight adjusted	75	71
8	5	Residential soil	Mass and weight adjusted	225	227
9	5	Residential soil	Mass and weight adjusted	675	685
10	8	Intravenous $Pb(Ac)_2 \cdot 3H_2O$	Weight adjusted	100	102

[a] Berm soil composite contained 14,200 ppm Pb while residential soil composite contained 3870 ppm Pb.

[b] Doses were based on group mean body weight adjusted every 3 days to account for animal growth. The amount of soil administered was adjusted for mass of Pb contained therein.

[c] Calculated as the administered daily dose divided by the measured or extrapolated daily body weight and averaged over Days 0–14 for each pig and over each group.

enteric bioavailability of Pb in young children necessitated selection based on similar physiological characteristics. Young pigs have similar physiology and have been used successfully as a model for gastrointestinal function of children (Dodds, 1982; Miller and Ullrey, 1987; Weis and Lavelle, 1991). Aspects of the pig model that make it uniquely attractive for bioavailability studies compared to rats and rabbits include: (1) the lack of coprophagy and the anatomical and physiological differences associated with this behavior; (2) the absence of complicating factors connected with the relatively high biliary excretion of Pb in rats; (3) the absence of rapid postnatal developmental changes in the active transport mechanism for Pb across the intestinal barrier of swine as seen in juvenile rats; (4) the similarity of immature swine in physiologic age and body weight to the childhood population; and (5) the ease of serial blood sampling without risk of anemia. The size and tractable nature of young pigs facilitates repeated blood sampling of ample volume (5–7 ml) for analysis and archiving, and for implantation of intravenous catheters.

MATERIALS AND METHODS

Test animals. Fifty Line-26 males (8- to 9-kg body wt) were selected from a group of 55 weanling boar pigs purchased from a Pig Improvement Co., Inc. (Franklin, KY) facility in Missouri. The extra 5 pigs were obtained to ensure that the 50 healthiest, most uniform in weight, animals entered the study; this extra group had 3 additional iv-implanted pigs to provide subjects with the least surgical complications. Dosing and care of the pigs were performed in compliance with the animal care and use protocol approved by the University of Missouri Animal Care and Use Committee in accordance with provisions of the "Guide for the Care and Use of Laboratory Animals," NIH publication No. 86-23 (1985). Pigs were ear-tagged for identification and placed in individual stainless-steel pens with wire floors and nipple waterers and fed a commercial pelleted swine ration (Super Pig, MFA Agri Services, Columbia, MO) for the first day of acclimation during a 7-day prestudy quarantine. Acclimation continued for 6 additional days during which the diet was gradually changed to 100% of a specially formulated low-lead (<0.2 ppm Pb) diet (Zeigler Bros, Inc., Gardners, PA) by Day −3. This nutritionally complete ration was fed in equally divided portions (half in the morning and half in the afternoon) at a daily rate of 5% of the mean body weight of all pigs in each group. This method of feeding was used to more accurately mimic intermittent feeding by children and to maintain a more uniform body weight between pigs within a group. Feed quantities and Pb doses were weight-adjusted every 3 days. In addition to daily visual inspection of the pigs, samples of blood were collected on Days −4, 7, and 15 for complete blood counts as a part of the swine clinical health-monitoring protocol. Indwelling catheters were surgically implanted in one group of five pigs for intravenous dosing.

Experimental design. Relative and absolute bioavailabilities of Pb in two composited test soils containing 3870 and 14,200 μg Pb/g of soil, respectively, were estimated by comparing them to an orally administered soluble Pb salt (lead acetate = $PbAc_2 \cdot 3H_2O$ = PbAc). Biological responses (area under the blood Pb concentration–time curve, and terminal liver–, kidney–, and bone–lead concentrations) produced by several doses of Pb from PbAc and lead-contaminated soil were measured. On Day −4 pigs were randomly assigned to 1 of 10 treatment groups (Table 1). Dosing was initiated on the morning of Day 0 and terminated on Day 14.

The amount of Pb in the soil that was as bioavailable as Pb from oral PbAc was calculated using the ratio of doses of Pb from $PbAc_2 \cdot 3H_2O$ to test soil that produced equivalent biological responses.

Collection and characterization of lead-contaminated soils. Study soils were composites from different areas of the Smuggler Mountain Superfund Site (US EPA's National Priority List) in Aspen, CO. The "berm soil" sample was a composite of nine sampling locations collected from a play area adjacent to a residential development (concentration = 14,200 μg Pb/g of soil). The "residential soil" sample was a composite of nine sample locations from residential properties (concentration = 3870 μg Pb/g of soil). Each soil was collected according to standard operating procedures and air-dried with minimal agitation at temperatures not exceeding 60°C. The soils were then sieved using nylon lead-free sieves. A 10-mesh sieve was placed on top followed by a 100-mesh sieve (<150 μm) and then the collection tray. The samples were gently tapped and swirled, not forced or ground, until smaller particles fell through the top sieve at which time the 10-

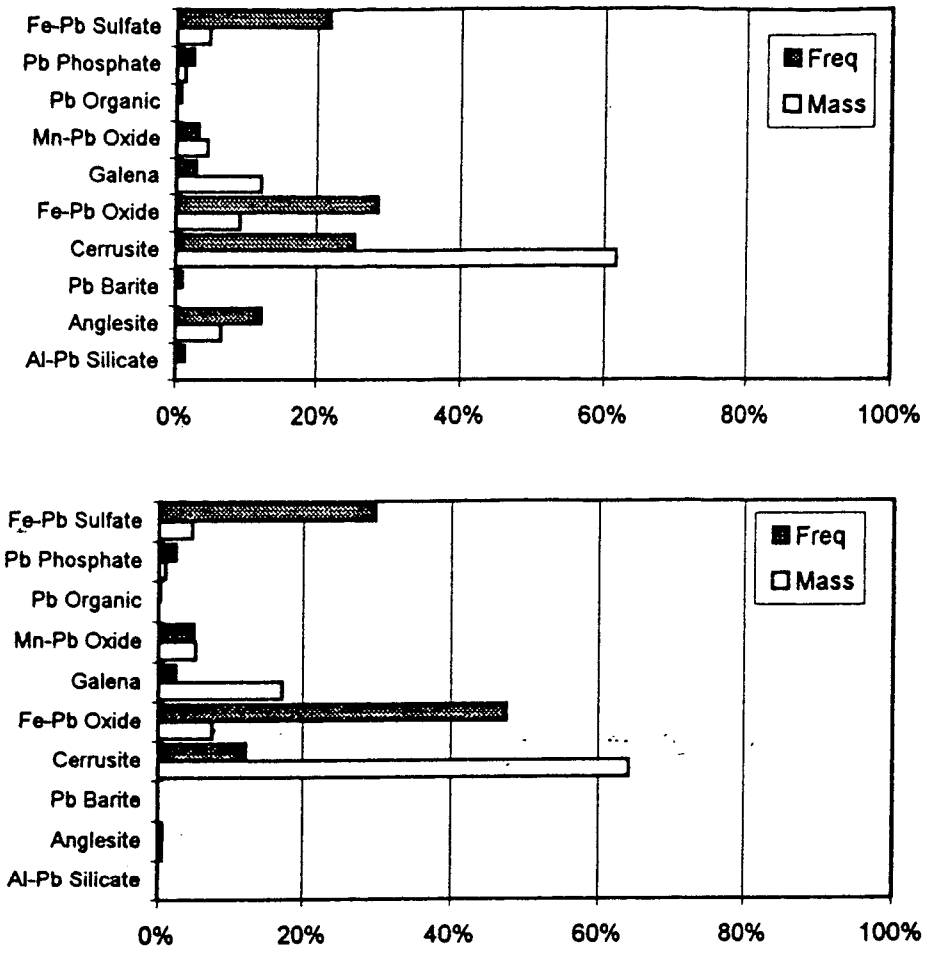

FIG. 1. Frequency of occurrence of lead-containing mineral types and the relative mass of lead contributed by each mineral type in berm (top) and residential (bottom) test soils.

mesh sieve containing the coarse soil fraction was removed and discarded. Tapping and swirling of the remaining 100-mesh sieve continued until the smallest particle fraction (<150 μm) was separated into the collection tray. This fraction was selected because particles less than 150 μm are most likely to adhere to the hands of children and be ingested by hand-to-mouth activity.

Test soils were initially analyzed using a field portable X-ray fluorescence instrument. Soil samples were prepared for bulk analysis using a KEVEX 0700 EDS (energy dispersive system). Quality control of sample analytical results was provided by use of a least squares calibration curve which was generated from a series of 12 EPA-certified standards. Soil characterization of the sieved fraction continued using an electron microprobe (JOEL 8600) equipped with four wavelength spectrometers, EDS detector, and data processing system. Grain mounts, 1 inch in diameter, were prepared using air-dried epoxy. Instrument calibration was achieved by use of standards of galena (PbS), angelsite ($PbSO_4$), and cerussite ($PbCO_3$). Test soil characterization endpoints included frequency of particles of each Pb mineral, particle size distribution of each mineral class, matrix of Pb-containing particles, and the total mass of lead present in each mineral form. In addition, the frequency of each type of particle existing in a liberated form, i.e., free from the surrounding matrix, was used to calculate the total relative lead form present in the samples.

Dosing, feeding, and sampling. Animals were dosed with lead for 15 days. To simulate intermittent childhood exposure, doses were divided equally and delivered daily starting at 9 AM and 3 PM, 2 hr before feeding in the morning (11 AM start time) and afternoon (5 PM start time). To facilitate precise dosing, feeding, and blood sampling, daily time details were followed with a 3-min interval allocated per pig for each procedure. For example, pig 506 was bled between 8:00 and 8:03 AM, dosed with the first half of the calculated daily dose between 9:00 and 9:03 AM, fed the first half of the calculated daily feeding (2.5% of group mean body weight) between 11:00 and 11:03 AM, dosed with the second half of the calculated daily dose between 3:00 and 3:03 PM, and fed the second half of the calculated daily feeding between 5:00 and 5:03 PM. This routine was followed sequentially for each pig on a daily basis for 15 consecutive days for all groups.

Calculation of doses and weight of feed was based on the group mean body weight adjusted every 3 days. Oral delivery of $PbAc_2 \cdot 3H_2O$ was achieved by placing a volume between 20 and 100 μl of an appropriate concentration of stock solution (5, 20, or 100 μg Pb/μl) into a depression in a 4- to 6-g mass of low-lead feed moistened with enough double-distilled water to form a dough-like consistency. After the $PbAc_2 \cdot 3H_2O$ solution was absorbed, the doughball was squeezed in on itself and hand fed. Pigs in each dose-group received the same volume of solution based on their respective group mean body weight. Similarly, dosing with the lead-contaminated soil was performed by placing the soil mass (±5% weighing precision) into the 4- to 6-g mass of moistened feed as previously described. Prior to removal of soil–dose aliquots, the approximately 1-liter bottles containing the bulk soil samples were gently mixed on a roller (U.S. Stoneware, East Palestine, OH) at 8 rpm for 30 min to ensure collection of homogenous soil samples.

TABLE 2
Metal Analysis of Test Soils

Analyte	Berm soil, concentration (ppm)	Residential soil, concentration (ppm)
Aluminum	5070	8440
Antimony	5.2	11.4
Arsenic	66.9	16.7
Barium	1640	1030
Beryllium	1.3	0.82
Cadmium	41.9	47.4
Calcium	37200	17300
Chromium	7.7	10.4
Cobalt	17.1	11.1
Copper	145	51.6
Iron	33700	23000
Lead	14200	3870
Magnesium	14300	6890
Manganese	2220	934
Mercury	0.77	0.23
Nickel	29.8	21.9
Potassium	1090	2140
Selenium	2	0.38
Silver	92.3	18.9
Sodium	249	114
Thallium	1.8	0.27
Vanadium	11.5	16
Zinc	6580	4110

The group dosed intravenously with $PbAc_2 \cdot 3H_2O$ solution (Grp10) received 100 μg Pb/kg/day in two equal doses (50 μg/kg/dose) by injecting 0.1 ml/kg of an aqueous solution containing 500 μg of Pb from $PbAc_2 \cdot 3H_2O$/ml. Doses were delivered with a Huber point needle into a subcutaneous vascular access port (Access Technologies, Skokie, IL) with attached indwelling venous catheter terminating in the anterior vena cava.

Blood samples for Pb analysis were collected 4 days before exposure (Day −4), on the first day of exposure (Day 0), and on Days 1, 2, 3, 5, 7, 9, 12, and 15 following initiation of exposure. Five- to 8-ml samples were collected by venipuncture from the anterior vena cava with needle and syringe and dispensed into collection tubes (Vacutainer, Becton Dickinson, Rutherford, NJ) containing potassium EDTA. To ensure adequate mixing of blood and anticoagulant, each tube was gently inverted six times. Sampling of blood on collection days began at 8 AM, 1 hr before the first of two daily doses of Pb, and 17 hr after the last lead dose from the previous day. This blood collection time was selected to minimize the kinetic rate of change in PbB concentration resulting from the preceding day's dose (LaVelle *et al.*, 1991).

Pigs were euthanized and necropsied on Day 15. Gross examination included evaluation of the brain, lungs, heart, liver, and the gastrointestinal and urinary tracts. Fifty to 100 g of the central liver lobe was removed from each pig and placed in a plastic bag (Whirl-Pak, Nasco), labeled, and stored at −70°C until preparation for Pb analysis. The right kidney was handled in like manner while the right femur was removed and flensed before storage.

Preparation and analysis of biological samples. One milliliter (±0.05 ml) of blood was pipetted into a polyethylene tube (Fisher Scientific, Pittsburgh, PA) followed by 9 ml of matrix modifier consisting of 0.2% v/v nitric acid, 0.5% v/v Triton X-100, and 0.2% w/v ammonium phosphate in double-distilled water. One-gram (±0.05 g) samples of kidney cortex or liver were placed in Teflon digestion containers with 2 ml of 70% nitric acid. Screw-caps were applied and containers were placed in a 90°C oven for 12 hr. Following digestion, container contents and rinsate were placed in a 10-ml volumetric flask and brought to volume with double-distilled water and then transferred to polyethylene tubes with screw-caps.

Whole right femurs were placed in Coors crucibles (Fisher Scientific, Pittsburgh, PA) and dried in an oven (Precision Scientific, Chicago, IL) at 100°C overnight. The dried bones were ashed in a muffle furnace (Barnstead/Thermolyne, Dubuque, IA) at 450°C for 48 hr and then ground into a fine powder with mortar and pestle. Two hundred-milligram aliquots were removed and dissolved in 10 ml of 1:1 (v/v) ultrapure nitric acid in double-distilled water. One milliliter of the dissolved bone solution was diluted to 10 ml in a polyethylene tube by adding 9 ml of bone matrix modifier consisting of an 853 ppm lanthanum solution derived from a stock solution. The lanthanum stock solution (1706 ppm lanthanum) was prepared by dissolving 2.0 g of lanthanum oxide in 250 ml of double-distilled water, adding 160 ml of ultrapure nitric acid brought to 1.0 liter volume with double-distilled water. The stock solution was used to prepare the 853 ppm solution as needed by mixing 1 vol with an equal volume of double-distilled water.

Samples for Pb analysis were submitted to the analytical lab in blinded fashion, except for identification of the sample matrix, by assigning each prepared sample a unique encoded number. A Perkin Elmer (Norwalk, CT) Model 5100 atomic absorption spectrometer with graphite furnace was used for Pb analysis. Quantitation of Pb in blood followed a modified method developed at the Centers for Disease Control and Prevention (Miller *et al.*, 1987).

Quality control and validation procedures. This study was conducted according to Good Laboratory Practice guidelines of the EPA (40 CFR 792). Quality control samples, including blanks, intra- and interlaboratory duplicates, spikes, and check samples from the Centers for Disease Control and Prevention, were prepared and assigned encoded numbers in the same fashion as test samples to mask their identity from analytical personnel. If spike recovery was outside the 80 to 120% acceptance range a second set was prepared and reanalyzed. Interlaboratory duplicates were analyzed by the metals laboratory at the Centers for Disease Control and Prevention. Dose verification also was performed on randomly selected samples. Internal quality assurance samples were analyzed every 10th sample, and the instrument was recalibrated after every 15th sample. A blank, duplicate, and spiked sample were analyzed with every 20 samples. The quantitation limit was defined as 3 times the standard deviation of a set of 7 replicates of a low-lead sample (2-5 μg/dl). The standard deviation ranged from 0.3 to 0.33 μg/liter, so the quantitation limit was 0.9–1.0 μg/liter (ppb). For blood (diluted 1/10), this corresponds to a quantitation limit of 10 μg/liter. For soft tissues, this corresponds to a quantitation limit of 10 μg/kg (ppb) wet wt, and for bone (final dilution, 1/500), the corresponding quantitation limit was 500 μg/kg ashed weight.

Leachates from containers, reagents, solutions, and equipment items were analyzed to ensure negligible Pb contamination of dosing materials or blood and tissue samples collected for analysis. Maximum acceptable concentration of Pb in animal drinking water was 10 μg/liter. Maximum acceptable increases in Pb concentration of the 2-ml leachates over acid blanks were: 5 μg/liter for blood tubes (Vacutainer, Becton Dickinson, Rutherford, NJ), 5 μg/liter for 15 ml blue-top polypropylene graduated tubes (Falcon tubes, Fisher Scientific, Pittsburgh, PA), 20 μg/liter for 10 ml polyethylene vials (Fisherbrand, Fisher Scientific), 20 μg/liter for teflon digestion vessels (Savillex, Minneapolis, MN), and 20 μg/liter for plastic bags (Whirl-Pak, Nasco). A 5-ml aliquot of each reagent or aqueous solution was placed in a Falcon tube for Pb analysis. Maximum acceptable Pb concentration for reagents was set at 5 μg/liter.

Data collection and analysis. The enteric absorption of lead and/or the biological response to absorbed lead (e.g., blood or tissue lead concentrations) has been shown to be linear or nonlinear functions of dose. Since equal biological responses are caused by equally absorbed doses of Pb, application of this general method yields consistent results for both linear and nonlinear dose–response curves; however, the converse is not true for nonlinear dose responses. Fundamental to this process was determining the ratio of dose of $PbAc_2 \cdot 3H_2O$ that induced the same response as some dose

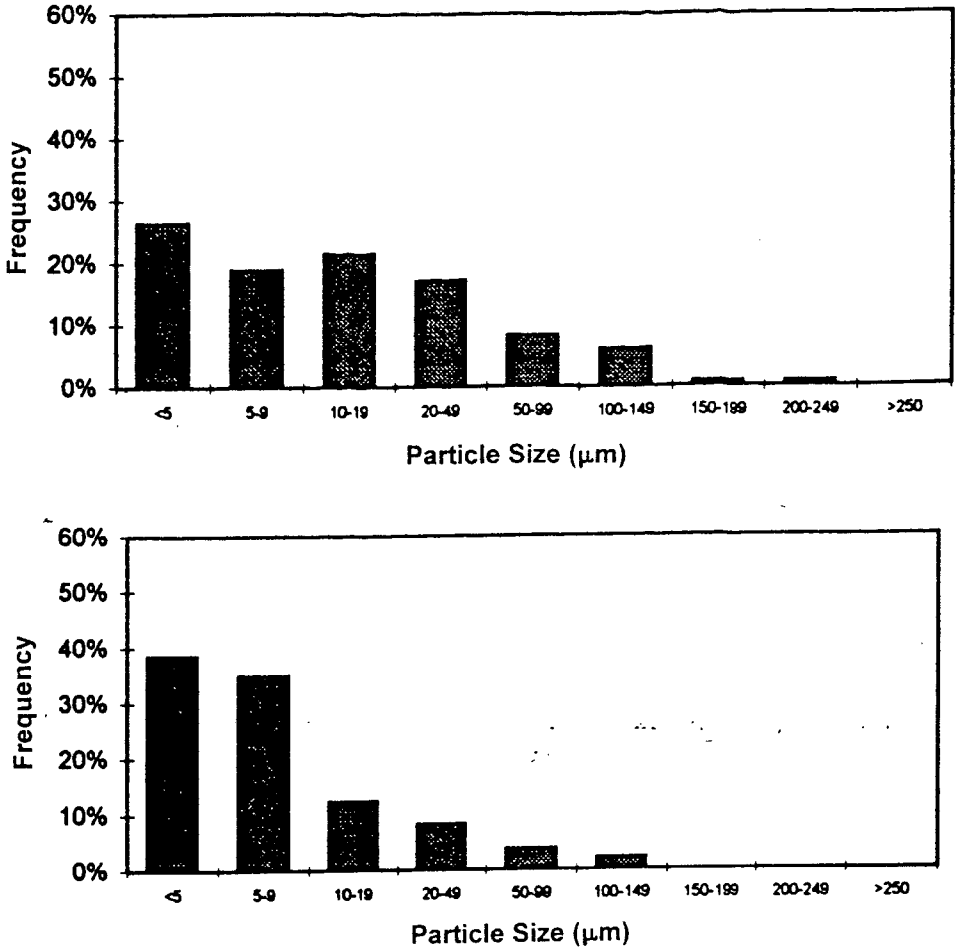

FIG. 2. Size distribution of lead-bearing particles in berm (top) and residential (bottom) test soils.

of test soil (Relative Bioavailability or RBA). For nonlinear dose responses, this was carried out by fitting mathematical equations to the PbB dose–response data of the $PbAc_2 \cdot 3H_2O$ reference compound and test soils using a curve-fitting program (Table Curve-2D, Jandel Scientific). Using responses of individual pigs, data from $PbAc_2 \cdot 3H_2O$ and lead-contaminated dose groups were fit to either straight-line (for tissues) or exponential (for PbB) curves. If one equation had a clearly superior fit as judged by the adjusted correlation coefficient (R^2) compared to the others, that equation was selected. Curve-fit parameters were constrained to exclude nonnegative values and data points outside the 95% prediction limits of the best-fit curve were excluded. Doses of $PbAc_2 \cdot 3H_2O$ and test soils inducing equivalent biological responses were calculated from these best-fit equations. Dose ratios of $PbAc_2 \cdot 3H_2O$-to-soil Pb inducing equivalent responses were then calculated for doses within the administered experimental dose range for each tissue. Estimated absolute bioavailabilities (ABA) were based on EPA's default assumption that lead in water has an assumed absolute absorption of 50%, so that

$$ABA_{soil} = ABA_{lead\ acetate} * RBA_{soil}.$$

Because the magnitude of variability in response between pigs in low-dose groups is large compared to the group mean response, the low-dose portion of the dose–response curves for lead-containing materials is particularly difficult to define with certainty. Because of the uncertainty in the low-dose portion of the dose–response curves, RBA values were not calculated for doses of test soil lower than the dose of lead acetate that yielded a response three times that of the mean of the negative control group. Similarly, if the response to the high dose of a test soil exceeded the response of the high dose of lead acetate, this introduced uncertainty in RBA values at high doses because calculations would require extrapolation of the lead acetate dose–response curve beyond the measured reference response data. To minimize this uncertainty, the lead acetate standard curve was not extrapolated beyond the highest measured individual response observed in pigs exposed to the high dose of lead acetate, and RBAs were not calculated for test soils at doses which yielded responses above this value. These uncertainties were minimized, since the linear-fits and constrained nonlinear fits produced constant RBAs over the experimental dose range.

RESULTS

Characterization of lead-contaminated soils. The most frequent lead-bearing particle types in both soils were iron–lead oxide, lead carbonate (cerrusite), and iron–lead sulfate (Fig. 1.). Of the total lead mass, most occurred in the form of cerrusite, with the remainder mostly lead sulfide (galena) and iron–lead oxide. Based on the measured frequency of particle types existing in the liberated state (i.e., lead exposed on the surface), of the calculated total lead in the samples,

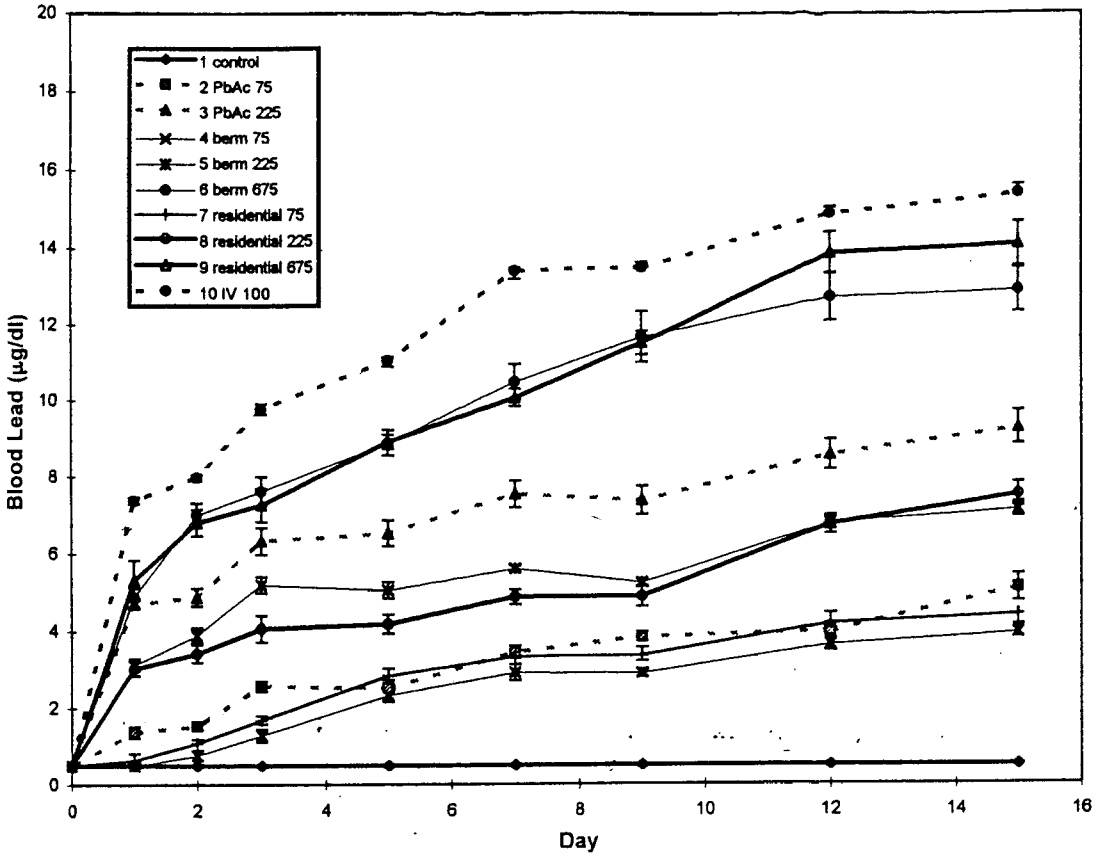

FIG. 3. Group mean blood lead (±SE) versus day of experiment in juvenile swine dosed with lead from lead acetate and lead-containing soils. Blood lead concentrations began below quantitation limits (1 µg/dl) in all groups 4 days before the start of dosing (Day −4), and the day of dose initiation (Day 0). Blood–lead values remained below quantitation limits throughout the study in control pigs.

about 33% existed in liberated particles in the berm soil sample, and 54% in the residential soil sample, mainly in the form of cerrusite with lesser amounts of iron–lead oxide. Other metals present in the test soils and their respective concentrations are detailed in Table 2.

Most of the lead particles in both samples were less than 10 µm in diameter (Fig. 2). These small particles are most likely to adhere to surfaces for transport and/or ingestion and provide larger surface areas leading to increased opportunities for solubilization.

Quality assurance. A randomly selected set of about 5% of all samples generated was submitted blind to the analytical laboratory for duplicate analysis. There was good intralaboratory reproducibility for blood and tissues, with linear regression lines having slopes of 0.99, intercepts of 0.09 for blood and 0.16 for tissues, and correlation coefficients of 0.99.

Analyses of check blood samples from the Centers for Disease Control and Prevention (CDCP) were lower than the nominal concentrations of 1.7 and 4.8 µg/dl. Analytical results for the 10 low check samples included 6 samples below the detection limit and 4 at 1 µg/dl. Results for the 10 high check samples ranged from 3.3 to 4.4 µg/dl (mean of 3.9 µg/dl).

For interlaboratory comparison, 15 whole blood samples were randomly selected from this study and sent to CDCP in blinded fashion for preparation and analysis. Similar to the check samples, blood concentrations reported by CDCP were an average 20% higher than our laboratory results. Reasons for this discrepancy are not apparent; however, calculated relative bioavailability (RBA) values are not affected. In particular, since both the lead acetate and test soil dose–response curves are biased by the same factor, calculation of the ratio (RBA) cancels any bias.

Animal health. Adverse effects from lead administration were not detected based on daily health evaluations, food consumption, and weight gain of the animals. All pigs consumed the low-lead diet readily, within 30 min or less of provision. The mean body weight of the pigs at the start of the 15 days of dosing was 9.4 kg (SE ± 0.02 kg) and at the conclusion was 16.1 kg (SE ± 0.03 kg).

Blood lead. In juvenile pigs orally dosed with either $PbAc_2 \cdot 3H_2O$ or soil–lead, the group mean blood–lead concentrations as a function of time increased rapidly within 1–2 days of dose initiation, and tended to plateau by the end of the study (Fig. 3). Blood–lead AUC dose–response data

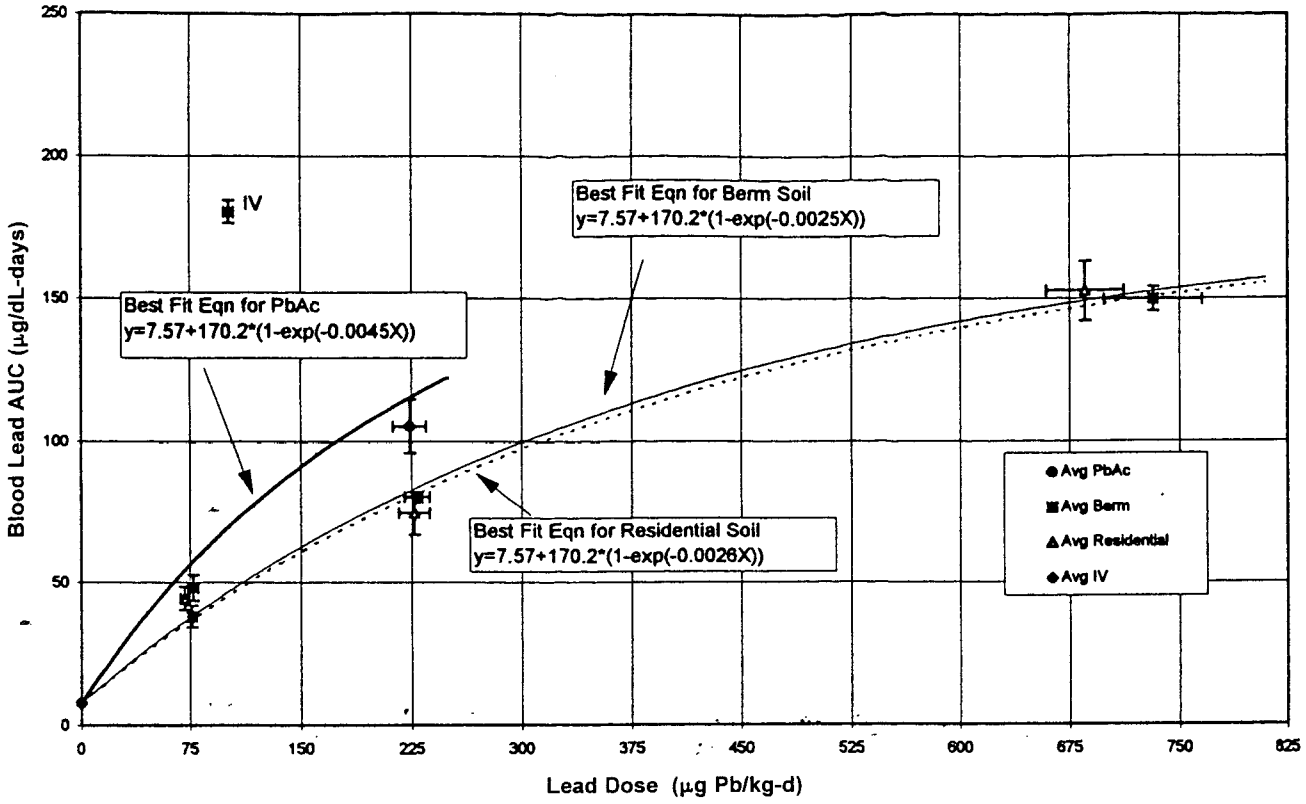

FIG. 4. Dose versus blood lead area-under-the-curve (AUC) for juvenile swine orally dosed daily for 15 days with lead from lead acetate (PbAc), berm, or residential soils. A single group of pigs was dosed intravenously (iv) with lead from aqueous lead acetate. Each data point represents the group mean dose and response, with the variability in dose and response shown by standard error bars.

were best described by an exponential equation (Fig. 4), which gave a consistent fit over repeated experiments.

Tissue dose response. The dose–response data for liver, kidney, and bone were best described by linear equations (Figs. 5–7) which also gave consistent fits over repeated experiments. The tissue-loading responses of liver, kidney (µg Pb/kg wet wt), and bone (µg Pb/g ash wt) to dosing with Pb from two contaminated test soils from the Smuggler Mountain NPL site are similar to each other and slightly lower than for the reference oral lead acetate.

Site-specific bioavailability determinations. RBA values were calculated for each test soil for blood, bone, liver, and kidney endpoints. The RBA values for blood–lead AUC and all three tissues were independent of dose (i.e., constant) for both test soils. The estimated RBAs from the blood–lead AUCs were 56% for the berm soil and 58% for the residential soil. For berm soil, estimated RBA values based on liver, kidney and bone, were 86, 68, and 72%, respectively. For residential soil, estimated RBA values based on liver, kidney, and bone were 74, 74, and 68%, respectively. The RBA best estimates (blood weighted 3:1 over each tissue) were then used to calculate absolute bioavailability (ABA) of lead in soil as follows:

$$ABA_{soil} = ABA_{lead\ acetate} * RBA_{soil},$$

Since EPA's current default ABA for a soluble form of lead (i.e., lead acetate) is 0.50 or 50%, the estimated ABA of lead in berm soil ranges from 28 (via blood AUC) to 43% (via liver uptake). Similarly, the estimated ABA of lead in residential soil ranged from 29 (via blood AUC) to 37% (via liver uptake).

Due to multiple measurements of PbB in each pig over time versus a single, terminal, tissue lead measurement (and because of the clinical relevance of PbB concentration in humans), the RBA for PbB was weighted at 75% versus 25% for tissues (i.e., blood weighted 3:1 over each tissue). This weighting method provided a "best-estimate" RBA of 63% for berm soil and 64% for residential soil. The corresponding ABAs of about 32% for use in EPA's Integrated Exposure Uptake Biokinetic model were slightly greater than EPA's default value of 30%.

DISCUSSION

This study advances the understanding of enteric lead absorption (bioavailability) from site-specific contaminated media using a juvenile swine model. Although there were significant differences in lead concentration (3870 ppm vs 14,200 ppm), both of the soils were relatively consistent in particle size, lead mineral type, and the associated matrix.

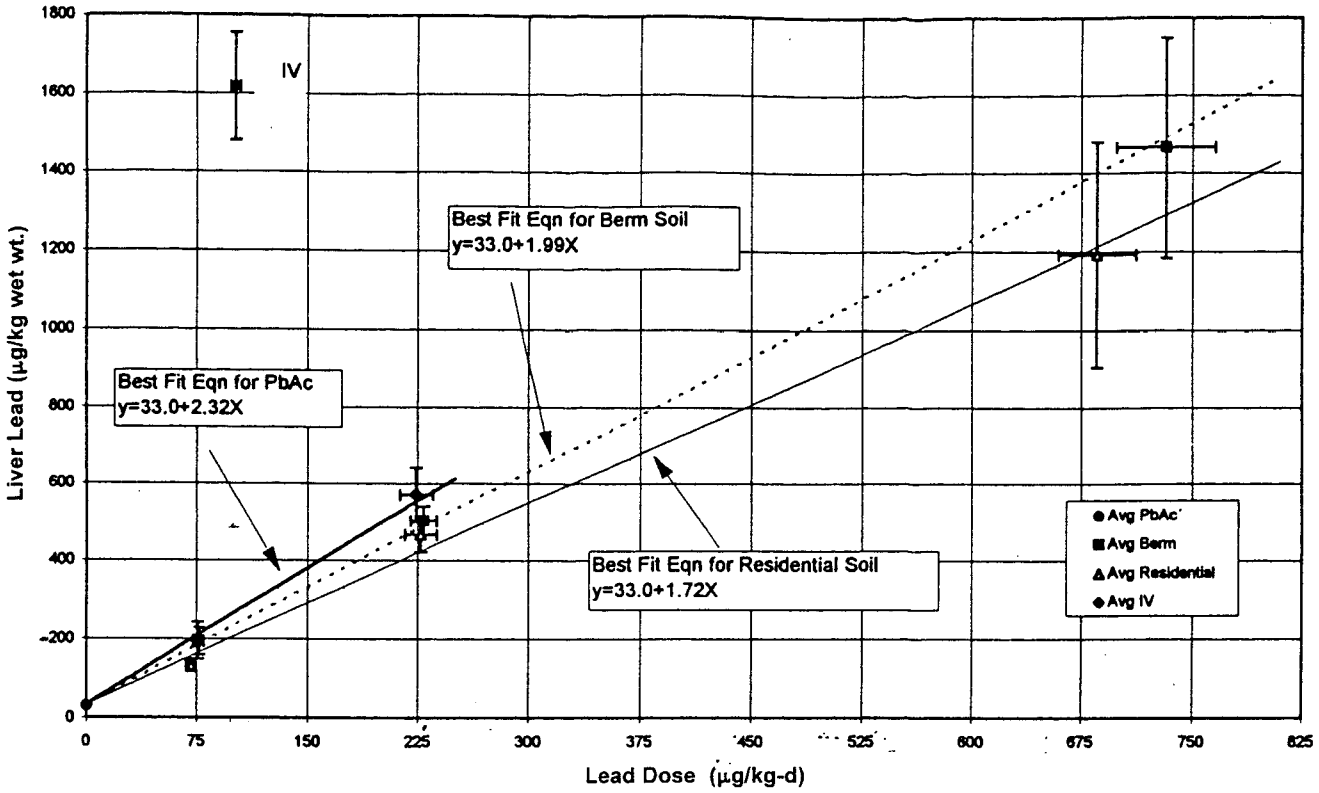

FIG. 5. Dose versus liver lead concentration for juvenile swine orally dosed daily for 15 days with lead from lead acetate (PbAc), berm, or residential soils. A single group of pigs was dosed intravenously (iv) with lead from a lead acetate solution. Each data point represents the group mean dose and response, with the variability in dose and response shown by standard error bars.

These factors were likely responsible for the similar bioavailability results.

The usefulness of these results stems from several factors including the juvenile swine model as a plausible surrogate for children and the method and range of dosing used in the experimental design. Some of the complications associated with past rodent studies on lead bioavailability are connected with the relatively high biliary excretion of Pb by rats (Castellino and Aloj, 1964; Klaassen and Shoeman, 1974; Gregus and Klaassen, 1986). Biliary excretion of lead by rats results in significant underprediction of lead bioavailability for mammals without such high biliary excretion. Physiologically relevant estimates of Pb bioavailability are key to adequate exposure and risk assessment in areas of ongoing childhood exposure. Furthermore, animal studies, conducted to assess bioavailability for the purpose of public health decision making, are most useful if they are designed to model human exposure at the appropriate developmental stage and plausible environmental dose. Rodent studies have been conducted using rats in older adolescent or adult developmental stages (Freeman *et al.*, 1992, 1994; Dieter *et al.*, 1993). These studies typically have assessed the absorption of Pb in rodent models at doses delivered in the diet that were substantially higher than those plausible for most environmental exposures. Excessive doses of soil containing high concentrations of other cations and minerals, in addition to minerals present in the diet, presumably could depress the absorption fraction of lead in rats as it does in humans (Blake *et al.*, 1983). Evaluation of lead bioavailability studies in rats by means of a physiologically based kinetic model revealed a reduction in the fractional absorption of lead as oral intake increased with the magnitude of this dose effect being lead-source dependent (Polak *et al.*, 1996). It is therefore likely that the low absorption rates measured in these and similar rodent studies are more a function not only of excessive doses, but also dose delivery in the feed coupled with the advanced developmental stage of the rats. In contrast, our work is intended to provide soil–lead absorption data that is more relevant to younger developing (nearer postweaning and sexually immature) mammals at smaller daily doses closer to those experienced by many chronically exposed children during a semifasted state (Weis and LaVelle, 1991).

For each test soil, bioavailability estimates (RBA and ABA) were calculated for blood (AUC), liver, kidney, and bone. For purposes of exposure assessment, there are several reasons why most emphasis should be placed on blood–lead concentrations (PbB). Blood–lead calculations are based on 10 measurements per animal over time, thereby enhancing the biological and statistical robustness compared to tissue calculations based on single measurements. The nonlinearity

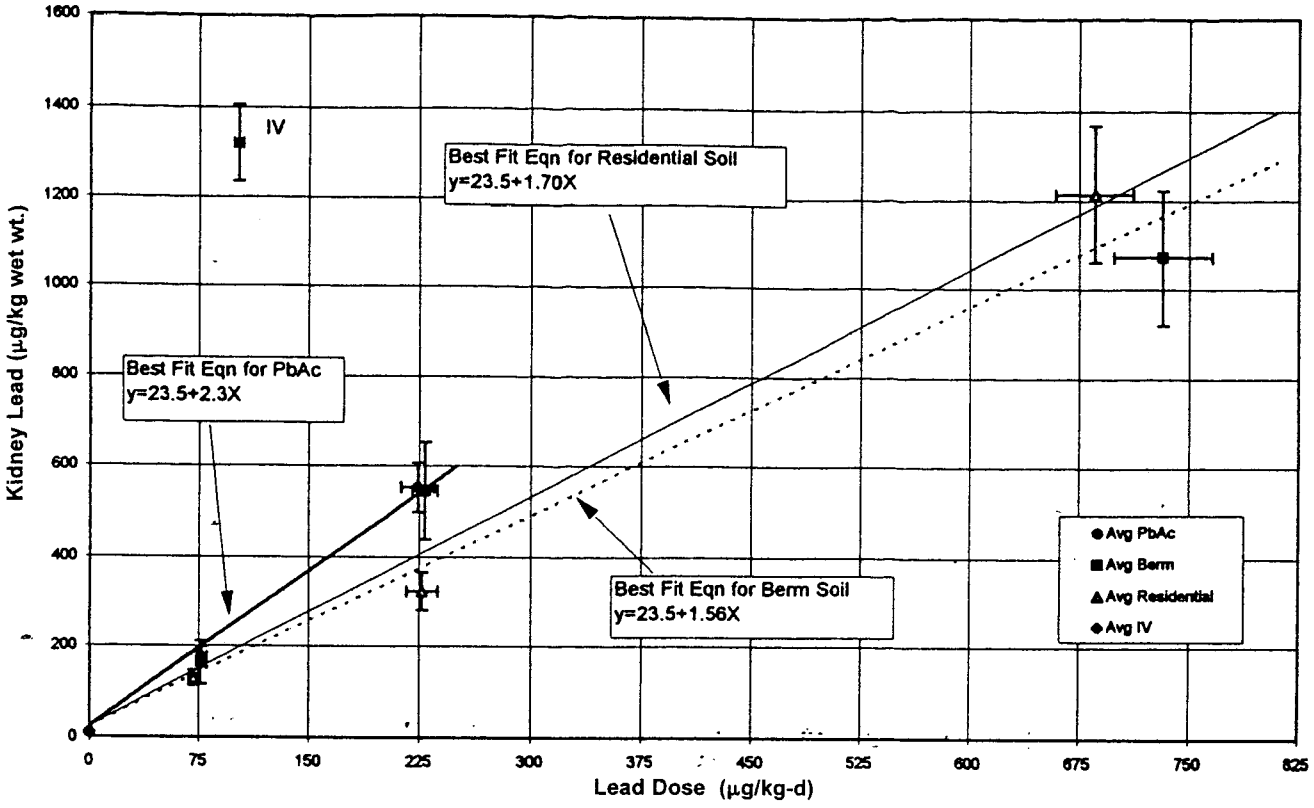

FIG. 6. Dose versus kidney lead concentration for juvenile swine orally dosed daily for 15 days with lead from lead acetate (PbAc), berm, or residential soils. A single group of pigs was dosed intravenously (iv) with lead from a lead acetate solution. Each data point represents the group mean dose and response, with the variability in dose and response shown by standard error bars.

of the dose–response curve for blood (Fig. 3) is similar to that observed in children (Sherlock and Quinn, 1986), while tissue–lead kinetics (linear in pigs) in humans are less certain. Blood–lead concentrations in children are thought to have nonlinear (cube root) relationships with water–lead concentrations (100 µg/liter) and dietary lead, with steadily smaller increases in blood lead as the amount of ingested lead increases (Sherlock and Quinn, 1986). Since blood is a comparatively homogeneous medium, obtaining a representative sample is uncomplicated. Consequently, the AUC endpoint (Fig. 4) is less susceptible to influence by random measurement errors, and associated RBA values are, therefore, more precise. Blood represents the common pathway linking and supplying all other tissues with lead absorbed from the gastrointestinal tract, and is also used routinely as a marker of central nervous system exposure in humans. However, data from end-loading tissue (e.g., liver, kidney, and bone) concentrations provide supplemental information on lead disposition and for comparison to blood loading.

The RBA values based on PbB AUC measurements (Fig. 4) are dose-dependent, tending to increase with dose of test soil. Values most relevant to the exposure assessment of humans are the RBA calculations at doses close to the range of childhood lead intake from soil. Ingestion of 100 mg of soil per day by a 10-kg child results in a daily intake of 10 µg Pb/kg per 1000 ppm Pb in soil, corresponding to doses of about 5–50 µg Pb/kg-day (or 10–100 µg Pb/kg-day for the 200 mg soil ingestion as a reasonable maximum exposure) for soil concentrations in the range of 500–5000 ppm. Relative bioavailability estimates from the AUC over this dose range are 56% (28% ABA) for berm soil and 58% (29% ABA) for residential soil in the juvenile pig model. These estimates of bioavailability for these particular lead-contaminated soils are close to EPA's default of 60% RBA (30% ABA).

In contrast to values based on PbB AUC, RBA calculations based on lead in tissues were apparently dose-independent; a consequence of the linearity of the dose–response curves for lead acetate and test soils. For berm soil, the RBAs based on kidney (68%) and bone (72%) are relatively close to that based on the blood–lead AUC (about 58%), while the RBA based on liver (86%) is considerably higher. The differences in RBAs between blood and tissues for the residential soil are less striking, a likely reflection of inherent model and experimental variability. The weighting of blood on a 3:1 ratio versus each tissue also tends to minimize the effect of a disparate single-point tissue response.

Increasing importance of quantitative risk assessment, and

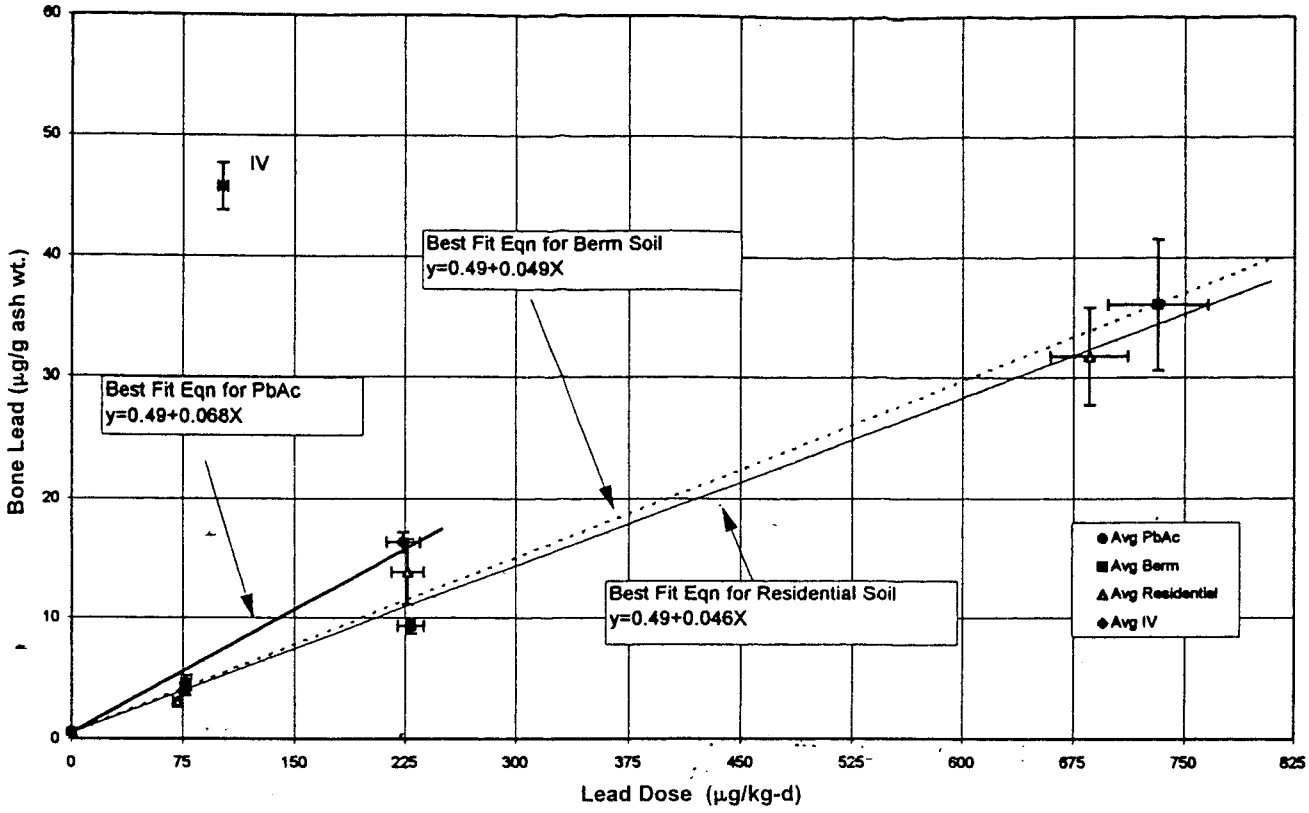

FIG. 7. Dose versus bone lead concentration for juvenile swine orally dosed daily for 15 days with lead from lead acetate (PbAc), berm, or residential soils. A single group of pigs was dosed intravenously (iv) with lead from a lead acetate solution. Each data point represents the group mean dose and response, with the variability in dose and response shown by standard error bars.

the associated regulations permitting some level of acceptable risk, emphasizes the necessity for greater confidence in these determinations and the need for accurate measurements of effective dose. The overall utility of the juvenile swine model lies in its ability to reduce the uncertainty associated with site-specific risk assessment of contaminated media, in general, by determining the relative bioavailability of lead (and possibly other toxicants) in a mammalian species closely related to children from a physiological basis.

ACKNOWLEDGMENTS

The authors thank Dr. Dan Paschal of the National Center for Environmental Health of the Centers for Disease Control and Prevention, Atlanta, Georgia, for providing quality control blood samples from the Blood Lead Laboratory Reference System.

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